

AIR LATERAL ROOT PRUNING AFFECTS LONGLEAF PINE SEEDLING ROOT SYSTEM MORPHOLOGY

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Abstract—Longleaf pine (*Pinus palustris*) seedlings were cultured with air lateral root pruning (side-vented containers, VT) or without (solid-walled containers, SW). Seedling root system morphology and growth were assessed before planting and 8 and 14 months after planting. Although VT seedlings had greater root collar diameter than the SW before planting, seedling height and ground line diameter were not affected by root pruning 14 months after planting. Root pruning did not affect seedling biomass. However, root pruning changed the patterns of biomass allocation. Before planting, VT seedlings allocated a greater percentage of biomass to roots and less to shoots than SW seedlings. Furthermore, VT seedlings allocated more root biomass to taproots at the expense of the first-order lateral roots (FOLR) and fine roots. This trend of favoring taproots by VT seedlings continued through 14 months after planting. For both types of seedlings, more than 50 percent of the FOLR originated in the top 2.5 cm of the taproot whereas greater than 70 percent of FOLR egressed below 7.5 cm of the root plug after planting. Fourteen months after planting, SW seedlings accumulated greater root biomass within the dimensions of the original root plug than VT seedlings. The VT seedlings had less extent of FOLR spiraling within the root plug than the SW seedlings before and after planting. Less FOLR spiraling and less root biomass increase within the root plug after planting in the VT seedlings may improve the physical stability of the VT longleaf pine saplings.

INTRODUCTION

Since the late 1980s, public, industrial, and private forest managers and land owners have been actively restoring the longleaf pine (*Pinus palustris* Mill.) ecosystem in the southeastern United States (Barnett 2002, Boyer 1989, Landers and others 1995). Container-grown longleaf pine seedlings are the preferred planting stock type because they have a higher first-year survival rate and a wider planting window than bareroot seedlings (South and others 2005 and references cited therein). For the 2005-2006 planting season in the southern United States, 70 percent of the longleaf pine seedlings produced was of the container type (McNabb and Enebak 2008). The demand for container stock longleaf pine continues to increase. However, one noted drawback of using container-grown seedlings for reforestation and afforestation is that the established saplings may succumb to wind-throw during high sustained winds (South and others 2001).

Improvements in the morphological quality of container stock root systems have been attempted by adding ridges to the container cavity or coating the cavity wall with a layer of low concentration copper compounds (Cu). Cavity ridges help reduce root spiraling by training primary lateral roots to grow vertically (Barnett and Brissette 1986). Slow release of low-concentration Cu from the cavity coating stops lateral roots from

elongating once they reach the cavity wall (Ruehle 1985). Copper root pruning increased taproot and secondary lateral root dry weights at the expense of primary lateral root dry weight in container-grown longleaf pine seedlings before planting (Sayer and others 2009, 2011). These Cu-grown longleaf pine seedlings were greater in size 5 years after planting than the non-Cu seedlings (Haywood and others 2012). Dumroese and others (2013) also reported that longleaf pine cultured in Cu cavities had greater root collar diameter and tap root biomass but less total root volume compared to the non-Cu seedlings. In a root growth potential test, longleaf pine seedlings cultured in Cu cavities produced more new roots than those grown in non-Cu cavities or bareroot seedlings (South and others 2005). Lodgepole pine (*P. contorta* Dougl.) grown in Cu cavities had fewer leaning seedlings 3 years after planting than those from the non-Cu cavities (Krasowski 2003).

In spite of these reported improvements in seedling root system architecture and field performance by Cu root pruning, reluctance to growing or planting Cu seedlings in forest industry still persists. It appears that this reluctance stems from the operational aspects of reforesting longleaf pine. For example, Cu seedling root plugs are less firm than the non-Cu root plugs; thus, harder to handle from lifting to planting (Sayer and

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others 2009). Furthermore, the Styrofoam Cu containers cost 2 cents more per cavity than the non-Cu Styrofoam ones. Compared to the plastic containers, the Styrofoam containers are two- to three-fold less expensive; but they have a shorter usable life (3 versus 10 years) and are harder to clean for reuse. Since it is impossible to coat the cavities of plastic containers with Cu, an alternative to the chemical pruning of lateral roots to improve root system architecture in the root plug is air pruning of the lateral roots. Here, we tested the effects of air lateral root pruning using side vents on plastic cavity walls on longleaf pine seedling stock quality and field performance, with emphasis on root system morphology. Seedlings cultured in plastic, solid-walled containers were used as the non-lateral root pruning control.

MATERIALS AND METHODS

Container-Grown Seedlings

Longleaf pine seedlings were obtained from two nurseries in early November 2013. Seed sources for these two groups of seedlings were not the same, but both were from Louisiana. The State Nursery in Monroe, LA, grew longleaf pine seedlings for 30 weeks without air lateral root pruning in plastic, solid-walled IPL Rigi-pots® IP110 cone-shaped containers (SW) with dimensions of 3.95 cm-12.7 cm-110 ml (cavity top diameter-depth-volume) and a density of 569 cavities m⁻². The International Forest Genetics & Seed Company (Moultrie, GA) grew longleaf pine seedlings for 31 weeks with air lateral root pruning in the Size 128 vented containers (VT) with dimensions of 3.8 x 3.8 cm-12.0 cm-110 ml (square top opening-depth-volume) and a density of 309 cavities m⁻². The interior 84 cavities (14 x 6) in the Size 128 container trays have vents on all sides with the top tier starting 6.0 cm from the cavity top and the bottom tier starting at 9.5 cm. Cavities on the outside of the container trays have vents on the sides facing inside. Both SW and VT containers have cavity ridges.

Field Experiment

The study site was on the Palustris Experimental Forest within the Kisatchie National Forest in Rapides Parish of central Louisiana (31°01'N, 92°37'W). The soils were a Malbis fine sandy loam (fine-loamy, siliceous, subactive, thermic Plinthic Paleudults), a Ruston fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Paleudults), and a Smithdale sandy loam (fine-loamy, siliceous, subactive, thermic Typic Hapludults). In November 2013, all longleaf pine seedlings were planted on the same day and within 3 days of receipt from the nurseries. In each of 4 plots, 6 rows were planted with 13 seedlings per row. Within each plot, three rows were randomly assigned to one of the two container types. Seedlings were planted with a Terra Tech Styro 5® dibble stick at 2 x 2 m spacing.

Laboratory and Field Assessments

Before planting, 20 seedlings of each root pruning treatment were randomly selected for destructive assessments of chlorophyll content, biomass, and root system morphology. Seedling root collar diameter and height were measured on all seedlings immediately prior to planting. Eight and 14 months after planting, one seedling from each row was randomly selected for the same destructive assessments as the pre-planting seedlings. The selected seedlings were excavated within a 30-cm radius of the stem and to a 30-cm depth in soil. Survival, height and ground line diameter were measured for the remaining seedlings in the field in January 2015.

Chlorophyll determination—Two needle fascicles per seedling were randomly selected from seedlings selected for pre-planting destructive sampling and for excavated seedlings. Needle surface area was measured with a displacement method by Johnson (1984). Chlorophylls a and b were extracted with N,N-dimethylformamide and the absorbance of the extract was read at 664 nm and 647 nm by a DU-70 spectrophotometer (Beckman Coulter Inc., Fullerton, CA) as described in detail by Sung and others (2010).

Biomass and root system morphology—Seedlings selected for pre-planting destructive sampling and the excavated seedlings were severed at the root collar. Growth variables, such as height, root collar diameter, and dry weights of needles, stems, and root systems were recorded. Root systems were carefully washed to remove growth media and soils. Root system morphology was assessed by placing a root system over a root plug template of corresponding container type. For seedlings before planting, only those primary lateral roots originating from the taproot, sturdy in structure, and with a diameter of at least 0.5 mm were counted as the first-order lateral roots (FOLR). For seedlings excavated 8 and 14 months after planting, primary lateral roots originating from the taproot within the original root plug and with a diameter of at least 0.9 mm were counted as the FOLR. For each FOLR, the depth of origination from the taproot, the depth of egress from the root plug, and the length within the root plug were measured.

To estimate the spiraling extent within the root plug for each FOLR, the portion of an FOLR between its originating point and first point of contact with the cavity wall was measured. This point was visually determined by finding the first deflection point on an FOLR where the orientation of the FOLR changed due to contact with the wall. The extent of FOLR spiraling within a root plug was estimated as follows:

For the FOLR that extended to the bottom end of the root plug:

Estimated FOLR spiraling extent (cm) = $L - DL - (RP - DP)$ [equation 1],

where

L (cm) = total length of a stretched FOLR, measured from the origination point on the taproot to the end of the FOLR within a root plug;

DL (cm) = deflection length, measured between the origination point of a stretched FOLR and its first contact point with the cavity wall;

RP (cm) = root plug length;

DP (cm) = depth of the first deflection point of a FOLR on the root plug.

For a FOLR that probably would egress (for seedlings before planting) or had egressed from the side of a root plug (for the excavated seedlings), [equation 2] was used.

Estimated FOLR spiraling extent (cm) = $L - DL - (EG - DP)$ [equation 2],

where

EG (cm) = depth of FOLR egress from the side of a root plug

Statistical Analysis

The study was a generalized randomized block design with four blocks each containing three replicates of two lateral root pruning treatments (VT and SW). Each of the replicates was a randomly positioned row of 13 seedlings with the same root pruning treatment. For analysis of non-destructive variables such as ground line diameter and height, the means of all surviving trees in a row were used in an analysis of variance (SAS Institute Inc. 2004). All of the destructive sampling variables such as dry weight and root system morphology variables were analyzed using a single subsample from each row. At planting time, 20 samples of all available seedlings for each root pruning treatment were selected and compared using a t -test. Significant treatment effects existed when $Pr > F$ (for the planted seedlings) or $Pr > t$ (for the not-planted seedlings) values were < 0.05 .

RESULTS AND DISCUSSION

Seedling Field Performance

Fourteen-month field survival of the SW and VT seedlings was 93 and 96 percent, respectively. A high survival rate is expected in container longleaf pine stock cultured in container cavities greater than 90 ml in volume (Dumroese and others 2009, Sung and others 2010). Lateral root pruning treatment did not significantly affect seedling height after 14 months in field with 5.3 cm (standard deviation (sd)= 0.8) for VT and 5.7 cm (sd= 0.6) for SW seedlings. No planted seedlings had emerged from the grass stage. The VT seedlings had significantly greater root collar diameter

at planting than the SW seedlings [8.6 mm (sd= 0.5) versus 7.2 mm (sd= 0.4)]. However, seedling ground line diameter did not differ between treatments 14 months after planting with 19.2 mm (sd= 1.9) for SW and 18.9 mm (sd= 1.9) for VT. These values were about 5 mm greater than the longleaf pine seedlings cultured in Cu and non-Cu cavities of comparable volume and 1 year after planted in central Louisiana (Sayer and others 2009).

Seedling Morphology and Biomass Allocation

Compared to SW seedlings, VT seedlings had a higher chlorophyll a and b content at planting, but not after 8 or 14 months in field (table 1). Fourteen months after planting, the chlorophyll content had doubled in SW seedlings and was within the range reported for second year planted longleaf pine seedlings (Sung and others 2010). The chlorophyll content may further increase in second year because needles were sampled in January. A decrease in chlorophyll content from 25.6 nmol cm⁻² in November to 14.5 nmol cm⁻² in December was found in second year field longleaf pine seedlings in central Louisiana (Sung and others 2010). Increases in needle chlorophyll content and total needle biomass in seedlings after 14 months in field (table 1) suggested that the root systems of these planted longleaf pine seedlings had acquired mineral nutrients from the soils to sustain the growth of new needles.

Seedling biomass increased more than 3-fold after 8 months in the field and almost doubled again during the subsequent 6 months for both SW and VT seedlings (table 1). Similar to the ground line diameter, seedling biomass was 5 to 11 g greater in this study than seedlings in the study of Sayer and others (2009). Seedlings in this study were cultured for 30 or 31 weeks whereas a culture period of 27 weeks was used by Sayer and others (2009) which resulted in smaller size seedling stock. This may partially explain the greater biomass accumulation in planted seedlings in this study. Although air lateral root pruning treatment did not affect seedling ground line diameter or total seedling biomass, it affected the biomass allocation patterns. Compared to VT seedlings, SW seedlings allocated more biomass to needles and less to root systems before planting and less to root systems 8 months after planting (table 1). This was in contrast to the results report by Sayers and others (2009). They did not observe differences in biomass allocation patterns between the Cu root pruning and non-Cu longleaf pine seedlings.

Root System Morphology and Root Biomass Allocation

The VT seedlings had greater root weight than the SW seedlings before planting (table 2). However, after 8 and 14 months in the field, total root weights were similar between these seedlings. The VT nursery stock allocated more biomass to the taproot and less to the

Table 1—Effects of side-vent air lateral root pruning on growth variables (mean±standard deviation) of container-grown longleaf pine seedlings sampled before planting (n=20) and 8 and 14 months after planting (n=12)

Type	Chlorophyll a and b	Root collar diameter ^a	Height	Seedling dry weight	Biomass allocated to		
	<i>nmol cm⁻²</i>	<i>mm</i>	<i>cm</i>	<i>g</i>	Needles	Stems	Roots
-----%-----							
Before planting (November 2013)							
SW	8.4±2.2 ^{bd}	6.9±1.3*	3.2±0.5*	4.3±1.1	62±6*	10±2*	28±5*
VT	11.4±1.4	7.9±1.0	2.9±0.5	3.9±0.8	41±6	16±3	43±6
Eight months after planting (July 2014)							
SW	9.9±4.9	14.4±1.9	3.8±0.8*	16.6±5.2	60±5	13±3	27±4*
VT	9.1±4.9	14.2±2.3	3.1±0.7	14.1±5.5	56±9	13±3	31±7
Fourteen months after planting (January 2015)							
SW	18.5±4.0	16.2±1.8	6.1±1.5	30.8±10.0	52±4	9±3	39±6
VT	15.7±2.9	16.0±2.3	6.0±1.7	27.9±11.8	54±4	10±2	36±3

Note: seedlings were cultured in side-vented containers (VT) for 31 weeks in Georgia or in solid-walled containers (SW) for 30 weeks in Louisiana. Seedlings were planted in Louisiana in November 2013.

^a Ground line diameter for planted seedlings.

^b For each variable within a sampling period, values associated with * have Pr > F or PR > t values of < 0.05.

FOLR compared to the SW stock (table 2). This trend of favoring taproot growth in VT seedlings continued through 14 months after planting (table 2). Apparently, lateral roots of the VT seedlings were air pruned during nursery culture. It was reported that longleaf pine seedlings cultured in Cu-coated cavities also showed greater root biomass allocation to taproots and secondary lateral roots at the expense of primary roots (Dumroese and others 2013, Sayer and others 2009). Although air pruning decreased the number of FOLR in VT stock at the time of planting, VT seedlings had a similar number of FOLR as the SW seedlings after 14 months in the field (table 2). There were block and container type interactions for FOLR number, diameter, and percent of biomass allocation to FOLR in seedlings 8 months after planting. Compared to the VT seedlings, SW seedlings had greater FOLR number, diameter, and biomass allocation except for seedlings in one block.

Air lateral root pruning treatment did not affect the depths of the FOLR origination from the upper 2.5 cm (table 2) or 5.0 cm (data not shown) of the taproots. More than 50 and 80 percent of the FOLR originated, respectively, from the upper 2.5 cm and 5.0 cm of the taproots. This pattern of FOLR origination was similar to the one found in container-grown longleaf pine seedlings 3 years after planting (Sung and others 2009). It was reported that 59 and 35 percent of FOLR originated in the upper 5.0 and 10.0 cm of taproots, respectively. The high density of FOLR near the top of the taproot cautions against a shallow planting strategy for container-grown longleaf pine stock. Seedlings planted too shallow would inadvertently leave the upper portion of the FOLR exposed after the growth media

disintegrated and render these roots at risk when a prescribed burn is introduced to the planting site later.

The spiraling estimating system used here showed that VT seedlings had less FOLR spiraling within the root plug even after 14 months in field (table 2). Although the cavity ridges trained most FOLR to elongate vertically within the root plug for both seedling types, the ridges did not eliminate FOLR spiraling completely. For example, a SW and a VT seedling with similar total root biomass had 3.7 cm and 1.2 cm FOLR spiraling, respectively (fig. 1).

Treatments did not affect the percent of FOLR egress below 7.5 cm of the root plug (table 2). More than 70 percent of FOLR egress below this zone (table 2). This result is in contrast to other studies that found root pruning using Cu coating increased the proportion of FOLR egress from the top portion of the cavity plug. For example, Sayer and others (2009) reported that Cu and non-Cu longleaf pine seedlings had, respectively, 46 and 19 percent of lateral roots egressed from the top 5 cm of the root plug. Sung and others (2009) showed that Cu-root pruning increased the percent of FOLR egress from the top 5.0 cm of the root plug from 26 to 48 percent in longleaf pine seedlings. In other words, the low percent of FOLR egress from the upper half of the root plug observed in the current study without a significant treatment effect indicates that air lateral root pruning only provided a limited improvement in the root system architecture. Sayer and others (2009) found a greater effect on first-year Cu-seedling growth by roots egressing into the top 5 cm of soils than those egressing from the bottom depth of root plug

and suggested these roots near the soil surface may increase soil resource acquisition and sapling vertical stability.

Both taproot biomass and number of FOLR number are important for seedling quality and field performance

for many tree species (Kormanik and others 1998).

Fourteen months after planting, both types of seedlings had similar number of FOLR (table 2) and seedling dry weight (table 1). After 14 months in the field, biomass of SW seedling FOLRs within the confines of the original root plug increased 11.89 g (from 0.43 g to 12.32 g);

Table 2—Root system characteristics (mean±standard deviation) of longleaf pine seedlings in response to side-vent air lateral root pruning during nursery culture

Type	Total root system	All egressed roots	Root biomass allocated to taproot	FOLRs ^a	FOLR diameter	Root biomass allocated to FOLRs	FOLRs Originating Upper 2.5 cm	FOLR Egress Below 7.5 cm	FOLR Spiral Length
	-----g-----		%	no.	mm	-----g-----	%-----		cm
Before planting (November 2013)									
SW	1.19±0.37 ^{ab}	-	26±8*	6.4±2.5*	0.79±0.17	10±5*	64±23	-	1.6±1.2*
VT	1.67±0.33	-	51±9	4.5±2.7	0.82±0.19	6±4	75±23	-	0.9±0.8
Eight months after planting (July 2014)									
SW	4.41±1.48	0.56±0.46	51±7*	10.5±2.6*	2.11±0.30*	31±8*	56±22	90±10	3.8±2.3*
VT	4.19±1.06	0.62±0.32	74±10	8.3±2.1	1.78±0.20	15±7	65±12	84±18	0.9±0.6
Fourteen months after planting (January 2015)									
SW	12.13±4.32	3.12±1.75	47±9*	9.9±2.7	3.39±0.51	40±8*	52±13	79±12	4.5±2.4*
VT	9.73±3.53	3.08±1.99	61±14	8.7±3.5	3.04±0.67	28±15	56±15	71±23	2.1±1.1

Note: seedlings were sampled before planting ($n=20$) and 8 and 14 months after planting ($n=12$). Seedlings were cultured in side-vented containers (VT) or in solid-walled containers (SW).

^aFOLRs = first-order lateral roots.

^b For each variable within each sampling period, values associated with * have $Pr > F$ or $Pr > t$ values of <0.05 .



Figure 1—(L) Root system of a longleaf pine seedling cultured in a solid-walled container cavity and excavated after 14 months in the field. Most of the first-order lateral roots egressed from the bottom of the confines of the original root plug (dashed blue line) and were spiraling. (R) Root system of a longleaf pine seedling cultured in a side-vented container cavity and excavated after 14 months in the field. Most of the first-order lateral roots egressed from the bottom of the confines of the original root plug (dashed blue line) and were not spiraling.

whereas biomass of VT seedling FOLRs within the root plug increased 7.58 g (from 0.23 g to 7.81 g). The faster growing and sometimes spiraling FOLR within the SW seedling root plug can exacerbate the FOLR spiraling problem and these FOLR may stragulate each other or the taproot later. The issue of longleaf pine sapling's vertical instability has been shown to be associated with root system architecture (such as lateral root spiraling and absence of taproot) and not necessarily with shoot growth (Sung and others 2009, 2012).

CONCLUSIONS

Longleaf pine seedlings cultured with air lateral root pruning in side-vented containers increased biomass allocation to the root system, especially to the taproot compared to those cultured without lateral root pruning in the solid-walled containers even after 14 months in the field. Furthermore, seedlings with root pruning had less extent of FOLR spiraling than the non-root pruning seedlings through 14 months in the field. Seedling root plugs from the vented containers did not create any difficulty for planting in this study. Since these seedlings were grown commercially, the extracting and handling at the nursery should be problem free. The air pruning treatment can be a solution to ease the reluctance to growing or planting longleaf pine seedlings cultured with chemical root pruning treatment such as Cu. Unlike the Cu root pruning, the treatment of air pruning of lateral roots did not change the vertical pattern of FOLR egress from the root plug after planting. More than 70 percent of the FOLR egressed into soil at a depth of greater than 7.5 cm. Further study is needed to test sapling vertical stability in longleaf pine with shallow or deep lateral rooting.

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